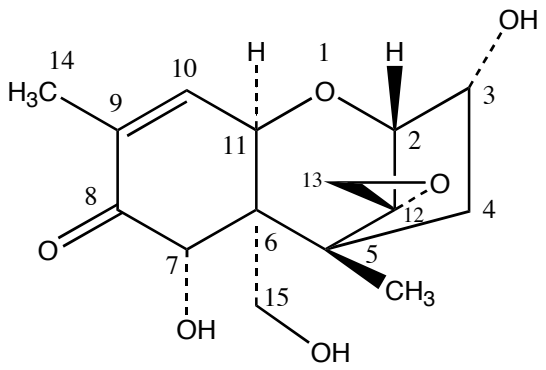
NTP Research Concept: Deoxynivalenol

Deoxynivalenol (Vomitoxin;12,13-Epoxy-3,7,15-trihydroxy-(3α,7α)-trichothec-9-en-8-one; CAS RN 51481-10-8)



Project Leader:
Dori Germolec, DIR/NTP/Toxicology Branch

Nomination Background and Rationale:
Deoxynivalenol (DON) is a trichothecene mycotoxin produced by certain Fusarium species that frequently occur in corn, wheat, barley, rice, and other grains in the field. DON was nominated by the National Institute for Environmental Health Sciences (NIEHS) for chronic toxicity and carcinogenicity studies and reproductive toxicity studies. The toxicological profile of DON has been described in a number of publications, yet definitive long-term studies are generally lacking. The widespread contamination of human foods and demonstrated toxicological activity of DON suggest a need for further studies to evaluate potential carcinogenicity and reproductive effects. These definitive studies will also provide necessary information for developing toxic equivalency factors (TEFs) to evaluate the aggregate risk from exposure to this and other tricothecene mycotoxins. In addition, the proposed studies will address data needs for DON that have been articulated by several groups, including the Joint FAO/WHO Expert Committee on Food Additives and the European Commission Scientific Committee on Food.

<u>Human Exposure</u>
Potential human exposure to DON may occur from consumption of contaminated oats, corn, wheat, barley, rice, and other field grains. DON has great stability during storage/milling and in the processing cooking of food, and does not degrade at high temperatures. DON has been detected in buckwheat, popcorn, sorghum, triticale, and other food products such as flour, bread, breakfast cereals, noodles, infant foods, pancakes, and malt and beer. Epidemiological studies have been conducted and acute toxicity, manifested as nausea, vomiting, diarrhea, headache, dizziness and fever were attributed to consumption of Fusarium-contaminated grains and the presence of DON at 3-93 mg/kg [0.01-0.31 mmol/kg]. In 15 urine samples collected from female inhabitants of Linxian County, China, a high-risk exposure region for DON and for esophageal

cancer, and Gejiu, a low risk region, DON was detected in all samples with mean concentrations of 37 and 12 ng/mL, respectively. DON was detected in the urine of 296 of 300 subjects whose cereal intake was monitored and urinary DON concentrations were significantly associated with cereal intake ($p < 0.0005$). The geometric mean concentrations were 6.55, 9.63, and 13.24 µg DON/day for low-, medium-, and high-cereal intake groups, respectively. Consumption of other grain-based foods such as breads, cereals, and pasta also were significantly associated with urinary DON concentrations In the Netherlands, 80% of one-year-old children exceeded the provisional maximum tolerable daily intake (PMTDI), and 20% of them had twice the value. Porridges were a significant source of DON intake in these children. A PMTDI of 1 µg/kg body weight (bw) was established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

There is the potential for occupational exposure among farmers engaged in grain threshing due to inhalation of pathogenic species of filamentous fungi and mycotoxins. High concentrations of fungi were noted in both grain and grain-dust samples during the threshing of cereals by a combine harvester. DON concentrations ranged from 0.015-0.068 mg/g [0.051-0.23 µmol/kg] in wheat grain samples.

Previous Toxicity and Carcinogencity Studies:

In a majority of studies, short-term and subchronic exposure to DON decreased body weight, weight gain, and feed consumption in rats and mice. In the most extensive DON feeding study reported to date, Iverson et al. (1995) examined the effects of feeding B6C3F1 mice 0, 1, 5, and 10 ppm DON for 2 yr and found significant body weight reduction in mice fed 5 and 10 ppm DON. Food consumption was not affected in females but was decreased for male test animals at the two highest doses. Full histopathologic analysis revealed that DON produced a dose-related decrease in liver preneoplastic and neoplastic lesions in males only which was attributed to reduced caloric intake. NOAELs for rodents were estimated to be 0.1–0.15 mg/kg bw/d, whereas for swine, NOAELs were 0.03–0.12 mg/kg bw/d.

DON appears to induce reproductive/teratogenic effects similarly in mice and rabbits with NOAELs of 0.5 and 0.6 mg/kg bw/d, respectively. However, there was little distinction between DON doses that produce maternal toxicity (feed refusal or reduced weight gain) and those that produce adverse reproductive effects, and a number of the studies that evaluated effects on reproduction were conducting using intraperitoneal injection as the route of exposure. Collins et al., 2006 examined fetal development in Sprague–Dawley rats treated with DON via oral gavage at doses of 0, 0.5, 1, 2.5, or 5 mg/kg body weight on gestation days (GD) 6–19. DON was considered a teratogen at 5 mg/kg day in this study based on the incidence of misaligned and fused sternebrae.

Toxicokinetic studies in B6C3F1 mice show rapid absorption of orally administered and instilled DON; elimination was biphasic. Limited toxicokinetics data was available for rats. Information on the bioavailabilty, mass balance, and metabolism was not available for the routes reported in the published toxicokinetics studies. However, based on the available data, there appear to be differences in toxicokinetics based on route of exposure and species.

In vitro studies suggest that DON does not induce gene mutations in bacterial or in mammalian cells in culture. However, published results from in vitro tests for induction of DNA damage or chromosomal aberrations in mammalian cell lines are consistently positive, suggesting DON may be clastogenic, and at very low concentrations. The experimental details are sparse for these studies but the consistency in results enhances their plausibility.

DON is a potent modulator of immune function and there is a significant body of literature describing both standard immunotoxicity testing and mechanistic studies which assess mode of action. No additional immunotoxicology studies are recommended.

Key Issues:
Although there is considerable data on the toxicity of DON, many of the studies, in particular the studies of effects on reproduction are difficult to interpret because of significant overt toxicity to the dam (manifested as decreased body weight and body weight gain) and inadequate experimental design. While there is adequate information on developmental toxicity, studies on the effects on fertility and reproduction need to be conducted with a careful attention to effects on males and females, dose and duration of exposure. In particular, there have been several studies that indicate effects on the male reproductive system, but no good assessment of how these may affect fertility. Dosed-feed studies may not be appropriate to separate the pharmacologic effects of DON from the effects of altered food consumption and may also be complicated by the limited availability of bulk quantities of purified materials for large-scale long-term toxicology studies. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has articulated critical research needs with regard to DON and other mycotoxins. The results of comparative studies of toxicity and toxicokinetics are needed to help to clarify species differences in sensitivity to DON. As the trichothecenes have similar toxic properties, albeit with different potencies, the JECFA recommended that toxic equivalency factors (TEF) be developed for the trichothecene mycotoxins, if sufficient data become available. Since humans may be exposed to a variety of mycotoxins in the diet, the establishment of TEF may be important in assessing aggregate risk. In view of the widespread human exposure to DON and the consistently positive results from *in vitro* genotoxicity studies, in vivo studies on genotoxicity are suggested, as well as a study of carcinogenicity in a second species (rat).

Currently, mills and processors in the United States and other countries conduct DON screening to divert grains exceeding established limits away from entering the human food supply. The level of this surveillance by producers, millers, and the food processing industry has increased sharply in recent years. Thus the possibility of high levels of DON sporadically entering human food is likely to have decreased substantially from 10 years ago.

Proposed Approach:

The overall goal of these studies is to characterize the reproductive toxicity, chronic toxicity and carcinogenicity of DON following oral exposure in rats. We propose a tiered approach to the research program. Tier one shall consist of prechronic dosed feed toxicity studies in Harlan Sprague Dawley rats. These studies will assist in the development of toxic equivalency factors for DON in the rat. Because of the issues with food consumption and decreased body weight gain following DON administration, these studies will also provide critical information needed to set doses for the RACB and 2-year carcinogenicity studies. A full toxicokinetic study in the rat is proposed for Tier one, in particular to provide information on bioavailability and tissue distribution. Toxicokinetic studies are also proposed for the B6C3F1 mouse, to provide comparative data that will address concerns with regard to species differences in sensitivity to DON. Finally, a stand-alone, acute genotoxicity study in rats and mice will be conducted to determine if DON has the potential for inducing chromosomal damage in vivo. DNA damage in liver, esophagus, and blood leukocytes will be assessed simultaneously in these studies using the comet assay.

Tier two shall consist of 2-year dosed feed toxicity and carcinogenicity study in the Harlan Sprague Dawley rat. Because DON is a developmental toxicant in the rat, the two-year studies will be conducted in adult animals, rather than as a perinatal study. This will avoid any limitation of dose in the cancer studies due to the developmental toxicity. A current guideline functional reproductive toxicology study in rats will be conducted to adequately address the potential effects on fertility and fecundity, based on the demonstrated evidence in the literature that the reproductive tract is a potential target for DON-induced toxicity.

Significance and Expected Outcome:
While there is adequate information on developmental toxicity, studies on the effects on fertility and reproduction need to be conducted with a careful attention to dose and duration of exposure. These studies will begin to address the research needs articulated by JECFA in 2001. JECFA has set a PMTDI of 1 μg/kg bw based on potential effects of DON on the immune system, growth, or reproduction. Additional toxicology studies may identify more sensitive endpoints relative to human exposures.

References:
Collins, T.F., Sprando, R.L., Black, T.N., Olejnik, N., Eppley, R.M., Hines, F.A., Rorie, J., and Ruggles, D.I. 2006. Effects of deoxynivalenol (DON, vomitoxin) on *in utero* development in rats. Food Chem Toxicol, 44(6):747-757.

Iverson, F., Armstrong, C., Nera, E., Truelove, J., Fernie, S., Scott, P., Stapley, R., Hayward, S., and Gunner, S. 1995. Chronic feeding study of deoxynivalenol in B6C3F1 male and female mice. Teratog Carcinog Mutagen, 15(6):283-306.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). 2001a. Deoxynivalenol. Safety evaluation of certain mycotoxins in food. WHO Food Additives Series No. 47. World Health Organization (WHO), Geneva—International Programme on Chemical Safety (IPCS). Internet address:

http://www.inchem.org/documents/jecfa/jecmono/v47je05.htm. Last accessed on March 16, 2004.